Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 8320–8324, December 1985
Biochemistry

Sequence-dependent energetics of the B–Z transition in supercoiled DNA containing nonalternating purine–pyrimidine sequences

(Z-DNA/DNA supercoiling/structural transition/topoisomers/statistical mechanics)

MICHAEL J. ELLISON†, RAYMOND J. KELLEHER III†, ANDREW H.-J. WANG†, JOEL F. HABENER‡, AND ALEXANDER RICH†

†Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139; and ‡Laboratory of Molecular Endocrinology, Massachusetts General Hospital, Howard Hughes Medical Institute, Boston, MA 02114

Contributed by Alexander Rich, July 31, 1985

ABSTRACT **The likelihood that a given DNA sequence will adopt the Z conformation in negatively supercoiled DNA depends on the energy difference between the B form and the Z form for that sequence relative to other sequences in the same molecule. This energy can be viewed simply as a sum of energies for the nearest-neighbor interactions within the sequence plus the energy required to stabilize the B–Z boundaries. Knowledge of these energetic terms would be of value in predicting when sequences become left-handed in response to negative superhelicity. Here we present an approach that can be used to determine the free-energy changes associated with all the nearest-neighbor interactions that can occur in Z-DNA. Synthetic stretches of d(C-G)$_n$ containing one or two transversions were cloned into plasmids, and the extent of the B–Z transition as a function of negative superhelicity was determined for each insert by two-dimensional agarose gel electrophoresis. By subjecting the data to statistical mechanical analysis, it was possible to evaluate the energetic penalty resulting from each base-pair (bp) substitution. Guanine to cytosine transversions cost 2.4 kcal (1 cal = 4.18 J)/(mol·bp), whereas guanine to thymine transversions cost 3.4 kcal/(mol·bp), to stabilize in the Z conformation. We have used these numbers, along with energetic values determined by others for the B–Z transition, to predict that certain strictly nonalternating purine and pyrimidine sequences may adopt the Z form readily.**

The positive free energy associated with the negative superhelicity of a closed circular DNA molecule is available for the facilitation of any process that involves helix unwinding. Because Z-DNA is of the opposite helical sense with respect to B-DNA (reviewed in ref. 1), the transition of a segment of DNA from the predominant B form to the Z form reduces the twist of the molecule, effectively removing negative supercoils. Therefore, the equilibrium between B-DNA and Z-DNA is shifted in the direction of the higher energy Z form by negative superhelicity. Thus far, only the sequences d(C-G)$_n$ and d(C-A)$_n$ have been unequivocally shown to adopt the left-handed conformation as a consequence of negative supercoiling (2–6). This can be attributed to the fact that the *syn* conformation is more favored for purine nucleotides than for pyrimidines (7), and in a sequence of alternating purines and pyrimidines, all the purines adopt the *syn* conformation, while all the pyrimidines remain in the *anti* conformation. This has led to the widespread view that purine–pyrimidine alternation is an adequate criterion for Z-DNA formation, regardless of base composition.

This view however, is an oversimplification of the problem. Stretches of alternating d(A-T)$_n$, for example, have not been observed to adopt the Z conformation. The matter is further complicated by several lines of evidence that have directly demonstrated that pyrimidines as well as purines can assume the *syn* configuration to form Z-DNA under certain conditions (8, 9).

The possibility that sequences without a regular alternation of purines and pyrimidines can adopt the Z conformation ultimately depends on the amount of free energy required to stabilize a pyrimidine nucleotide in the *syn* conformation and its opposing purine nucleotide in the *anti* conformation. If the energy is low enough, it is reasonable to expect that sequences that bear little resemblance to a strict alternation of purines and pyrimidines can adopt the Z conformation in a negatively supercoiled molecule. These energies can be viewed in a simple thermodynamic context. We can assume that the energy required to stabilize a given base pair (bp) in the Z form depends only on that base pair and its immediate neighbors. The quantity of energy required to effect the B–Z transition within a given sequence can then be described as the sum of the energetic terms for the nearest-neighbor interactions that comprise that sequence plus the energy necessary to stabilize two B–Z junctions. In a negatively supercoiled molecule, the stored free energy may then be partitioned between superhelix formation and the stabilization of the interactions occurring in Z-form DNA, thereby reducing the problem to a statistical mechanical one.

It has been previously demonstrated that the extent of Z-DNA formation in the sequences d(C-G)$_n$ and d(C-A)$_n$ as a function of negative superhelicity can be understood in terms of a simple statistical mechanical model (4, 10). In one study, the amount of energy required to stabilize a base pair in the sequence d(C-G)$_n$ in the Z form, as well as the energy required to stabilize the B–Z junctions, were determined (4). Here we use these values, and a similar approach, to determine the energetic cost for base-pair changes that disrupt the alternation in d(C-G)$_n$. The values we obtain for these base-pair changes are used to predict that certain nonalternating sequences may flip to the Z form relatively easily in response to the free energy of negative supercoiling. The approach that we use here could be logically extended to determine the energy required to stabilize all of the nearest-neighbor interactions that could occur in Z-DNA.

MATERIALS AND METHODS

Preparation of Plasmid Topoisomers. A population of topoisomers was prepared for each plasmid by adding various amounts of ethidium bromide to aliquots of plasmid in the presence of topoisomerase I as described (2).

Two-Dimensional Gel Electrophoresis. Agarose gel electrophoresis was performed on a horizontal submersible slab gel apparatus. Gels were formed from 200 ml of 1% agarose in

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: bp, base pair(s).

TBE buffer (90 mM Tris borate/2.5 mM EDTA, pH 8.3) in a 20 × 24 cm gel mold. A single sample well (diameter, 1/16 inch) was placed in the corner of the gel 1 cm from each edge. DNA (0.5–1.0 μg) was loaded onto the gel in 4 μl containing 30% sucrose and 1% bromophenol blue. Gels were electrophoresed in the first dimension at room temperature in the presence of 2300 ml of TBE buffer at 110 V for 22 hr. The gel was then equilibrated in 2500 ml of TBE buffer containing chloroquine phosphate at 0.75 μg/ml for 8 hr with gentle agitation. The gel was electrophoresed in the second dimension at room temperature using 2300 ml of the same buffer for 20 hr at 80 V. The gel was stained for 2 hr in 1 liter of distilled water containing 1 μg of ethidium bromide per ml, and then it was destained for 2 hr against 2 liters of distilled water. Gels were then illuminated with short-wave ultraviolet light and photographed with Polaroid Land Film (type 55).

Synthetic Oligodeoxyribonucleotides and Plasmid Construction. Oligodeoxyribonucleotides were synthesized by the phosphoramidite method. The four oligodeoxyribonucleotides synthesized were as follows: (Z1a) 5′ d[GATC$(CG)_8$ $GG(CG)_7$], (Z1b) 5′ d[$GATC(CG)_7CC(CG)_8$], (Z2) 5′ d$(CG)_{12}$, and (Z3) 5′ d[$(CG)_3A(GC)_5T(CG)_3$]. Z1a and Z1b were annealed to each other and cloned into the *Bam*HI site of pBR322. The self-complementary oligonucleotides Z2 and Z3 were annealed to the duplex form and cloned into the *Pvu* II site of pBR322. Plasmids were sequenced as described (11). It is worth noting that the number of plasmid isolates with the correct insert sequence was low (10–20%). The reason for this is not fully understood. In the case of Z2, an isolate with the correct sequence was never obtained. One of the plasmids isolated, however, contained an insert with the sequence 5′ d[$(CG)_6GG(CG)_5$], which proved to be valuable for the experiments described herein.

RESULTS AND DISCUSSION

Measuring the Extent of the B–Z Transition. The DNA sequences of the inserts contained in the four pBR322-based plasmids used in this study are shown in Fig. 1. The plasmid pLP32 contains 32 bp of alternating d(CpG) cloned into the *Bam*HI site (2). Plasmid pZ1 is identical to pLP32 with the exception of a single guanine to cytosine base-pair substitution in the middle of the Z-forming insert. Plasmids pZ2 and pZ3 contain Z-forming inserts 24 bp long cloned into the *Pvu* II site. The ends of the *Pvu* II site contribute a 5′ guanine and a 3′ cytosine, making the effective length of the Z-forming inserts 26 bp. Like pZ1, pZ2 and pZ3 contain base-pair changes that disrupt the alternating d(CpG) character of the sequence. pZ2 contains a single guanine to cytosine base-pair change in the middle of the d(CpG) stretch, while pZ3 disrupts the alternation with two guanine to thymine transversions separated by 10 bp.

Two-dimensional agarose gel electrophoresis has proved to be a particularly effective means of measuring the extent of the B–Z transition in good Z-forming inserts as a function of negative superhelicity (4, 5). The negative superhelicity of a closed circular DNA is a consequence of a deficit in the linking number of the two strands, α, with respect to that of the theoretically relaxed configuration, α_0°. The number of superhelix turns has been defined in terms of this altered linkage: $\tau = \alpha - \alpha_0^\circ$ (12, 13). Any change in the linking number (Lk) of a DNA is distributed topologically between its duplex twist (Tw) and its axial writhe (Wr) in the following way (14):

$$\Delta\alpha = \Delta Lk = \Delta Tw + \Delta Wr. \quad [1]$$

The net unwinding resulting from a reduction in linking number, expressed quantitatively as $\alpha - \alpha_0^\circ$, is thus partitioned between the torsional and bending components of the helix axis, in a manner that is dependent on the environment. It is the writhe, negative by convention in an underwound molecule, that determines the hydrodynamic properties of the molecule and permits electrophoretic separation of topological isomers. Increasing the net difference of the linking number from the relaxed state produces corresponding changes in the writhe, thereby increasing the rate of migration of the plasmid. The parameter α_0° can then be defined as the population average linking number of topoisomers that have the least mobility in a gel (unlike α, α_0° is not necessarily integral).

Increasing the linking difference of a closed circular DNA also increases its free energy relative to the relaxed state. This stored energy is termed the free energy of supercoiling:

$$\Delta G_\tau = K(\alpha - \alpha_0^\circ)^2, \quad [2]$$

where K is dependent on the length of the DNA at constant temperature (15, 16). In a topoisomer distribution, at some negative value of $\alpha - \alpha_0^\circ$, enough free energy is stored in the molecule to produce a detectable amount of Z-DNA in the test sequence under examination. In a given topoisomer, the linking number is constant in the absence of strand scission, and the fluctuations in twist and writhe are coupled (i.e., $\Delta Tw = -\Delta Wr$). Therefore, the reduction in twist resulting from Z-DNA formation produces a corresponding decrease in the molecule's writhe, equivalent to the removal of a defined number of negative supercoils, bringing about a reduction in electrophoretic mobility.

The presence of topoisomers with positive and negative supercoils as well as topoisomers that have undergone the B–Z transition within the insert makes the interpretation of a gel run in one dimension difficult. It is possible, however, to unambiguously assign the extent of the B–Z transition in a given test sequence to specific topoisomers by running the gel in a second dimension in the presence of the unwinding intercalator, chloroquine, a condition that does not favor Z-DNA formation.

Fig. 2 shows a typical two-dimensional gel produced by simultaneously electrophoresing topoisomers from plasmids pZ2 and pZ3. Mixing experiments such as these permit a more accurate comparison of the extent of transition in plasmids that differ from each other by only a few base-pair changes. Plasmid pZ2 differs from pZ3 by only 3 bp in the Z-forming insert. Corresponding topoisomers for both plasmids that have not undergone the B–Z transition migrate identically and are superimposed in the gel. At a linking difference of approximately −15, however, differences in the migration of the two plasmids become noticeable. pZ2 is the

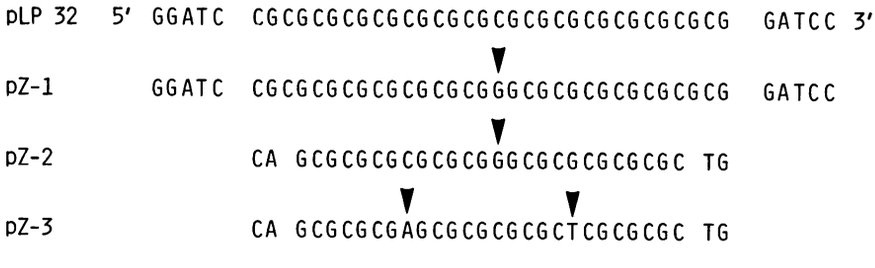

FIG. 1. Z-forming plasmid inserts. Arrows mark positions of base-pair changes that disrupt the alternation of the sequence. In pLP32 and pZ1, the flanking base pairs are derived from the *Bam*HI site of pBR322. The flanking base pairs of pZ2 and pZ3 are derived from the *Pvu* II site of pBR322.

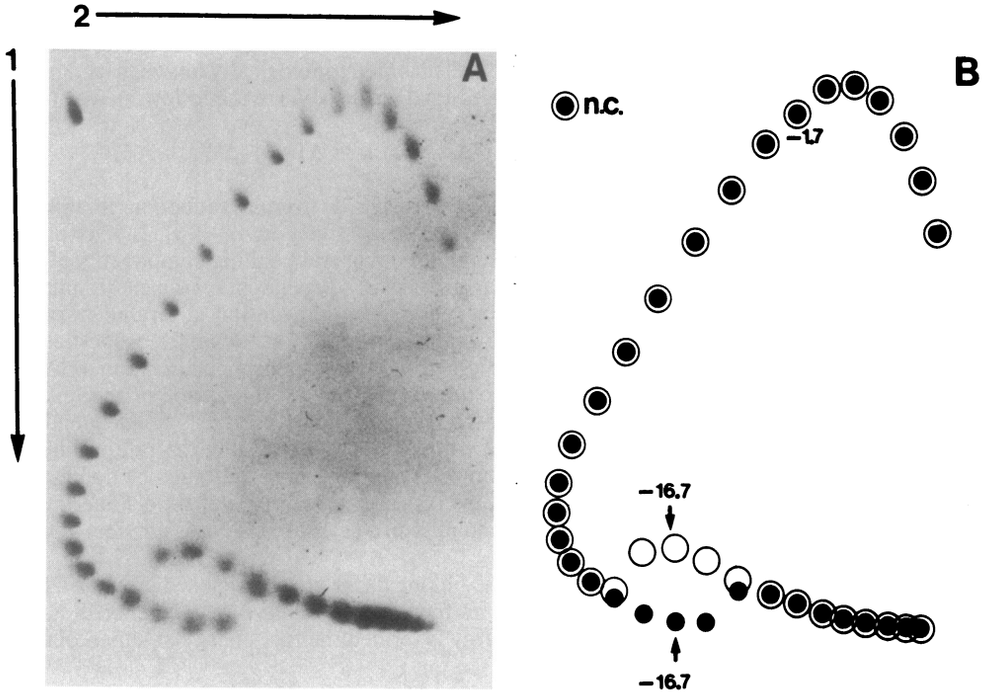

FIG. 2. Two-dimensional gel electrophoresis of mixed topoisomer populations from two plasmids with different transversions in the Z-forming insert. (*A*) Photograph of the actual gel. (*B*) A schematic diagram of the gel in *A*. Open circles represent topoisomers from pZ2; closed circles indicate the positions of topoisomers from pZ3. n.c., Form II (nicked circular) plasmid. The negative linking difference $-(\alpha - \alpha_0^\circ)$ for selected topoisomers is also shown.

first to undergo the transition, as indicated by the retarded mobility of topoisomers with a linking difference of −15.7 to −17.7. At a linking difference of −16.7, the insert of pZ2 is essentially all in the Z form. In comparison, pZ3 completes the transition three topoisomers later, at a linking difference of −19.7. Upon completion of the transition, topoisomers of both plasmids once more become superimposed.

Three or four two-dimensional gels were run for each of the four plasmids, and unwinding data as a function of negative superhelicity were accumulated. Some of the gels involved mixing experiments similar to the one shown in Fig. 2; other gels involved separate electrophoresis of each plasmid. The data from these experiments are best visualized by plotting the average reduction in twist observed for a particular test sequence ($-\langle\Delta Tw\rangle$) as a function of the negative linking difference of each topoisomer $[-(\alpha - \alpha_0^\circ)]$ (4). Plots of this kind for the four test sequences examined are presented in Fig. 3. It should be noted that the total reduction in twist observed with each insert is compatible only with a transition to a left-handed helical conformation.

Statistical Mechanical Analysis of Unwinding Data. The change in twist produced in $d(C\text{-}G)_n$ sequences as a function of negative superhelicity can be described by a statistical mechanical treatment derived from the zipper model for the helix–coil transition in polypeptides and DNA (4, 17, 18). In summary, the configuration partition function for the two-state B–Z transition in a chain composed of identical units is given by the expression

$$Q = 1 + \sum_{k=1}^{n} (n - k + 1)\sigma s^k, \qquad [3]$$

where $(n - k + 1)$ represents the number of ways of arranging k consecutive dinucleotides in the Z form in a sequence of n dinucleotides, σ is the nucleation constant, and s is the propagation constant. This latter parameter takes the form $s = \exp(-\Delta G_{B\text{-}Z}/RT)$, in which $\Delta G_{B\text{-}Z}$ represents the free energy change for the B–Z conversion of the kth dinucleotide. The reference state for a given test sequence is the B form. Nucleation of Z-DNA is assumed to occur at any point within the sequence with a free-energy change of that required to stabilize two B–Z junctions. Propagation then gives rise to a single block of k dinucleotides in the Z form within a sequence of $2n$ nucleotides, where k ranges from 0 to n. The net contribution of the free energy due to supercoiling (Eq. 2) is used to evaluate the partition function for each value of the linking difference $\alpha - \alpha_0^\circ$ for the plasmid, within a specified range. From this, it is possible to calculate the average change in helical twist, $\langle\Delta Tw\rangle$, from Z-DNA formation as a function of the change in linking number, $\alpha - \alpha_0^\circ$.

With this model, five parameters are sufficient for the prediction of the unwinding effects of a stretch of strictly alternating d(CpG), $2n$ nucleotides long, as a function of changing linking number: (*i*) the free energy of supercoiling, ΔG_τ, associated with each topoisomer; (*ii*) the degree of helical unwinding, a, associated with the conversion of a dinucleotide in the B form to the Z form; (*iii*) the degree of unwinding, b, occurring at the B–Z junctions (*iv*) the energy required for the stabilization of a dinucleotide in the Z form, $\Delta G_{B\text{-}Z}$; and (*v*) the energy required for the stabilization of two B–Z junctions, ΔG_J. The relationship between ΔG_τ and linking differences has been determined (Eq. 2). The degree of unwinding resulting from a dinucleotide in the Z form is calculated from the helical repeats of B-DNA and Z-DNA, as determined from crystallographic data and solution studies (4, 19). Values for the remaining three parameters, b, $\Delta G_{B\text{-}Z}$, and ΔG_J, were determined by Peck and Wang under conditions identical to those used here (4).

In the present analysis, the five parameters described above are held constant, and a sixth parameter, the energy required to stabilize the dinucleotide containing the base substitution, $\Delta G^*_{B\text{-}Z}$, is varied until the best fit is obtained between the experimental unwinding data and the calculated unwinding as a function of $\alpha - \alpha_0^\circ$.

The Energetic Penalty Associated with Transversions in $d(C\text{-}G)_n$. pZ1 differs from pLP32 by a single guanine to cytosine transversion occurring in the middle of the 32-bp stretch of d(CpG). The effect of this base substitution on the formation of Z-DNA as a function of superhelicity is evident from Fig. 3*A*. The energetic cost of this change that best fits the unwinding data for pZ1 is 2.4 kcal (1 cal = 4.18 J)/(mol·bp). pZ2 also contains a single guanine to cytosine transversion in the middle of a stretch of d(CpG). pZ2 differs from pZ1, however, in both the length and the position of the test sequence in the plasmid. Despite these differences, the unwinding characteristics of the test sequence of pZ2 can be

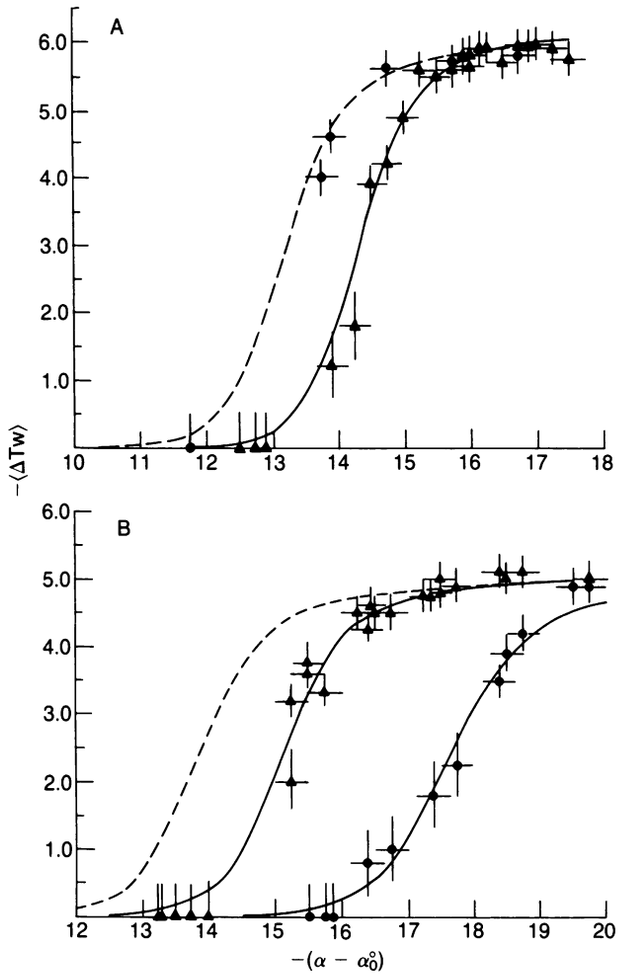

FIG. 3. Z-DNA formation as a function of negative superhelicity. Unwinding data accumulated from two-dimensional gels for each plasmid are seen here plotted as a function of the negative linking difference $-(\alpha - \alpha^{\circ}_0)$. Curves have been calculated from the partition function described in the text. (*A*) ●, pLP32; ▲, pZ1. (*B*) ▲, pZ2; ●, pZ3. In all cases, $\Delta G_{B-Z} = 0.66$ kcal/(mol·dinucleotide) and $\Delta G_J = 5.0$ kcal/(mol·junction), as determined previously (4). In plasmids containing transversions, ΔG°_{B-Z} has been varied to obtain the best fit to the data. Here, $\Delta G^{\circ}_{B-Z} = 2.4$ kcal/(mol·transversion) for pZ1 and pZ2, and 3.4 kcal/(mol·transversion) for pZ3. These values are estimated to be accurate to within ±20%. In *B*, the broken line represents the theoretical B–Z transition for $d(C\text{-}G)_{13}$.

likewise described by assigning 2.4 kcal/(mol·bp) to the energetic cost of stabilizing the transversion in the Z conformation (Fig. 3*B*). The fact that the B–Z transition of both of these test inserts can be described by an identical set of energetic parameters demonstrates that differences in the flanking sequences between pZ1 and pZ2 do not have a measurable effect on the stability of reasonably long stretches of d(CpG). This observation does not mean that the energy required to stabilize the B–Z junctions at the boundary between the insert and the flanking regions is sequence independent; it merely illustrates that the statistical weight of these junctions is small in comparison to junctions present within the stretch of d(CpG).

In contrast to the above findings for guanine to cytosine substitutions, an analysis of pZ3 (Fig. 3*B*) indicates that the energetic cost of guanine to thymine substitutions is 3.4 kcal/(mol·bp). The reason that guanine to thymine substitutions demand a higher energetic price than do guanine to cytosine substitutions is unclear at the present time. The inability of energy-minimized models *in vacuo* to shed light on these differences suggests that the discrepancy arises from environmental factors, such as solvent and ionic interactions. It is interesting to note that increasing the A + T content of alternating purine–pyrimidine stretches decreases the Z-forming potential of the sequence. $d(C\text{-}G)_n$, for example, forms Z-DNA more easily than $d(C\text{-}A/G\text{-}T)_n$, while $d(T\text{-}A)_n$ has not yet been found to adopt the Z form. In view of the observation that guanine to thymine transversions are more costly than guanine to cytosine transversions, it can be argued that A+T-rich DNA sequences in general are less likely to become left-handed, regardless of the sequence arrangement.

Nearest-Neighbor Interactions. Unlike B-DNA, the structural repeat unit of Z-DNA is the dinucleotide. Therefore, there are two classes of nearest-neighbor interactions in Z-DNA, regardless of nucleotide sequence: 5′ *anti–syn* and 5′ *syn–anti*. There is no reason to believe that these two types of interactions will be energetically equivalent. In fact, the substantially different types of stacking geometries that give rise to the dinucleotide repeat in Z-DNA suggest that these values may be quite different (19).

The energy values determined here for base-pair substitutions that disrupt the sequence alternation of $d(C\text{-}G)_n$ can be viewed as the sum of energetic parameters for these two types of nearest-neighbor interactions. In the case of guanine to cytosine transversions, 2.4 kcal are partitioned between two types of CpC nearest neighbors. In one type, the conformation of the bases is 5′ *anti–syn*; in the other type, the arrangement is 5′ *syn–anti*. For guanine to thymine transversions, 3.4 kcal is partitioned between CpT and TpC, where the common thymine is in the *syn* conformation, and the cytosines are both *anti*. The values obtained here and by others (4, 10) are summarized in Table 1 as a set of free-energy differences between the B and Z forms for the nearest neighbors shown.

As a consequence of the alternating structure of Z-DNA, a given sequence in the Z form could be composed of as many as 20 different nearest-neighbor interactions for the DNA duplex. With the approach taken here, it is possible to determine the energetic cost of each one of these interactions. Such a study would require the construction of 20 plasmids, each carrying a different Z-forming insert of constant length, which would differ from a control sequence, such as $d(C\text{-}G)_n$, by only a few base pairs.

Table 1. ΔG_{B-Z} for certain nearest-neighbor interactions

	Syn/anti	*Anti/syn*	ΔG_{B-Z}, kcal/mol	Ref.
1.	5′ -G C- 3′ 3′ -C G- 5′	5′ -C G- 3′ 3′ -G C- 5′	0.33	4
2.	-A C- -T G-	-C A- -G T-	0.66	10
3.	-C C- -G G-	-C C- -G G-	1.2	This work
4.	-T C- -A G-	-C T- -G A-	1.7	This work

Shown here are the average free energy changes for the B–Z transition associated with certain nearest-neighbor interactions. The values listed correspond to the averages for the two types of interactions shown in each row. The interactions shown in rows 3 and 4 arise as a consequence of transversion in $d(C\text{-}G)_n$. Because the values for ΔG_{B-Z} given in the text represent the sum of both types of interactions shown in rows 3 and 4, they are twice as great as those listed here. The values in rows 1, 3, and 4 were determined under identical conditions of pH and ionic strength. The value in row 2, however, came from data obtained in a 40 mM Tris acetate buffer (5).

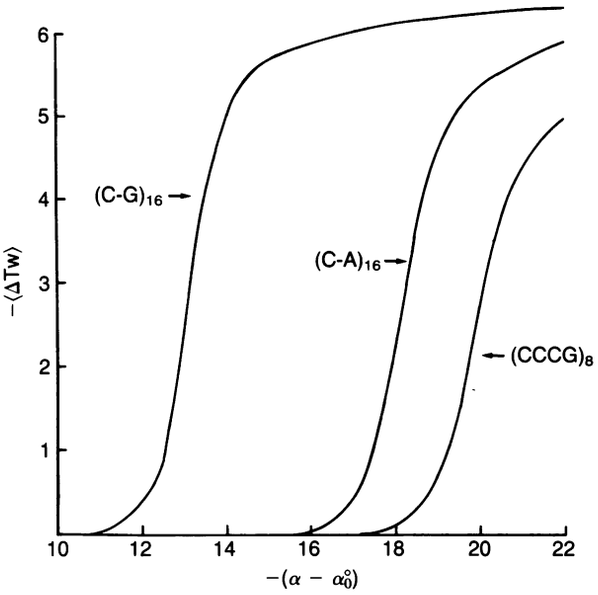

FIG. 4. Prediction of the supercoiling-dependent B–Z conversion of the sequence $d(CCCG)_8$. For comparison, transition curves for $d(C\text{-}G)_{16}$ and $d(C\text{-}A)_{16}$ are also shown.

Predicting the Z-Forming Potential of Other Sequences. The energetic values presented in Table 1 can be used in the statistical mechanical model used here to predict the Z-forming potential of a variety of sequences as a function of negative superhelicity. The sequences that can be chosen for analysis are subject to two limitations. A fully rigorous approach demands that they be composed entirely of the nearest neighbors shown in Table 1. Furthermore, because each energetic parameter represents an average of two nearest-neighbor interactions, a given sequence must contain an equal proportion of the nearest neighbors that correspond to a specific $\Delta G_{B\text{-}Z}$ value.

Implicit in this predictive approach are two additional assumptions. First, the free energy required for the formation of the B–Z boundaries, ΔG_J, is taken to be sequence independent. This assumption warrants further investigation. Second, it is assumed that the propensity of the analyzed sequence to adopt the Z form is such that partitioning of ΔG_τ to the formation of underwound structures elsewhere in the molecule can be neglected. Although this is an oversimplification for sequences requiring a relatively large amount of energy to form Z-DNA, it appears to be a fair approximation for strong Z-forming sequences, where competition from other transitional equilibria in the plasmid is minimized.

Taking the above limitations into consideration, we have modeled the B–Z transition for the sequence $d(CCCG)_8$ in the context of a plasmid the size of pBR322 (4363 bp). This sequence is of some interest because it does not possess the alternating purine–pyrimidine character of other sequences that have been observed to adopt the Z form readily. The predicted unwinding of $d(CCCG)_8$ as a function of $-(\alpha - \alpha_0^\circ)$ is shown in Fig. 4. For comparison, plasmids of equal size containing the good Z-forming sequences $d(C\text{-}G)_{16}$ and $d(C\text{-}A)_{16}$ have been included in this analysis.

Interestingly, the midpoint of the transition of $d(CCCG)_8$ occurs at a linking difference only two turns removed from the midpoint of $d(C\text{-}A)_{16}$. From this analysis, then, it is predicted that the B–Z transition of the sequence $d(CCCG)_n$ is only slightly more costly than that of $d(C\text{-}A)_n$. The predicted transition for $d(CCCG)_8$ occurs over a range of topoisomers that can be measured readily by two-dimensional gel electrophoresis. Experiments designed to directly test this prediction are currently under way.

It has been proposed that sequences with strong Z-forming potential can act as hot spots in genetic recombination (20–23). The presence of sequences in the genome that do not alternate in purines and pyrimidines but can nonetheless form Z-DNA at relatively low superhelix densities greatly expands the number of sites capable of participating in such Z-DNA-dependent processes.

We acknowledge Gary Gryan and Chon Vo for their assistance in the preparation of oligonucleotides. This research was supported by grants from the National Institutes of Health, the American Cancer Society, the National Aeronautics and Space Administration, the Office of Naval Research, and the National Science Foundation. M.J.E. was supported by a fellowship from the Medical Research Council of Canada. R.J.K. was supported by funds from the Undergraduate Research Opportunities Program at Massachusetts Institute of Technology.

1. Rich, A., Nordheim, A. & Wang, A. H.-J. (1984) *Annu. Rev. Biochem.* **53**, 791–846.
2. Peck, L. J., Nordheim, A., Rich, A. & Wang, J. C. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4560–4564.
3. Singleton, C. K., Klysik, J., Stirdivant, S. M. & Wells, R. D. (1982) *Nature (London)* **299**, 312–316.
4. Peck, L. J. & Wang, J. C. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 6206–6210.
5. Haniford, D. B. & Pulleyblank, D. E. (1983) *Nature (London)* **302**, 632–634.
6. Wang, J. C. (1984) *J. Cell Sci. Suppl* **1**, 21–29.
7. Haschemeyer, A. E. V. & Rich, A. (1967) *J. Mol. Biol.* **27**, 369–384.
8. Wang, A. H.-J., Gessner, R. V., van der Marel, G., van Boom, J. H. & Rich, A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 3611–3615.
9. Feigon, J., Wang, A. H.-J., van der Marel, G. A., van Boom, J. H. & Rich, A. (1985) *Science* **230**, 82–84.
10. Vologodskii, A. V. & Frank-Kamenetskii, M. D. (1984) *J. Biomol. Struct. Dynam.* **1**, 1325–1333.
11. Maxam, A. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–560.
12. Vinograd, J. & Lebowitz, J. (1966) *J. Gen. Physiol.* **49**, 103–125.
13. Wang, J. C., Peck, L. J. & Becherer, K. (1983) *Cold Spring Harbor Symp. Quant. Biol.* **47**, 85–92.
14. Fuller, F. B. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 815–819.
15. Pulleyblank, D. E., Shure, M., Tang, D., Vinograd, J. & Vosberg, H.-P. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4280–4284.
16. Depew, R. E. & Wang, J. C. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4275–4279.
17. Schellman, J. A. (1958) *J. Phys. Chem.* **62**, 1485–1494.
18. Flory, P. J. & Miller, W. G. (1966) *J. Mol. Biol.* **15**, 284–297.
19. Wang, A. H.-J., Quigley, G. J., Kolpak, F. J., Crawford, J. L., van Boom, J. H., van der Marel, G. & Rich, A. (1979) *Nature (London)* **282**, 680–686.
20. Slighton, J. L., Blechl, A. E. & Smithies, O. (1980) *Cell* **21**, 627–638.
21. Klysik, J., Stirdivant, S. M. & Wells, R. D. (1982) *J. Biol. Chem.* **257**, 10152–10158.
22. Haniford, D. B. & Pulleyblank, D. E. (1983) *J. Biomol. Struct. Dynam.* **1**, 593–609.
23. Kmiec, E. B., Angelides, K. J. & Holloman, W. K. (1985) *Cell* **40**, 139–145.